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FULL PAPER

Ramification of pH in pectinase-assisted extraction on the antioxidant capacity of Arabica spent coffee ground (SCG) extract

[1]Shukri, N.A., [1]Mohd Zin, Z.,[1]MohdMaidin, N., [2]Hasmadi, M. and [1,*]Zainol, M.K.

[1]Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Mengabang Telipot, 21030, Kuala Nerus, Terengganu, Malaysia
[2]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

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Abstract

Spent coffee ground (SCG) is the by-product of coffee processing that is produced up to 45% in coffee beverage and instant coffee processing and is believed to contain high amounts of antioxidants. Despite the fact that SCG has exerted many advantages, the information on obtaining the antioxidant values using enzymes are still scarce. The objective of the study was therefore to determine the total phenolic and flavonoid content of antioxidant activity and antimicrobial activity in Arabica SCG extracted using pectinase at different pH values. Arabica SCG was extracted using pectinase at pH 3, pH 4, pH 5, pH 6 and pH 7 and analyzed for its anti-microbial activity and antioxidant properties (DPPH, FRAP, total phenolic and total flavonoid content along with individual flavonoids using HPLC). Arabica SCG was extracted using pectinase at pH 3, pH 4, pH 5, pH 6 and pH 7 and analyzed for its antimicrobial activity and antioxidant properties (DPPH, FRAP, total phenolic and total flavonoid content along with individual flavonoids using HPLC). The result showed that the antioxidant capacity of the SCG extract at pH 4 exhibited higher DPPH and FRAP values. The total phenolic and flavonoid content exhibited in (1.38±4.42, mg GAE/g sample and 22.57±0.27 mg QE/g of dry sample). Flavonoids namely quercetin, kaempferol, rutin, gallic acid, catechin, epigallocatechin gallate, p-coumaric acid and myricetin were present in all samples at various levels. The SCG in pH 7 extract showed the highest concentrations of the individual flavonoid compound in the sample and the highest inhibition zone on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Thus, the optimum pH of the pectinase which is pH 4-5 had provided the highest yield in antioxidant capacity and activity of the Arabica SCG extract with this extraction method that can be used for food preservation as well as in the nutraceutical industry.

1. Introduction

Coffee is a brewed drink that was introduced in New York City in the mid-1600s and is one of the most widely consumed drinks made from roasted coffee beans around the world (Zhou and Hyppönen, 2019). The high and growing demand for coffee has led to large quantities of by-products such as silver-skin coffee, spent ground coffee (SCG), coffee husk and coffee-cut stem to the environment. SCG is the by-product or residue in the process of coffee powder treated with steam or hot water in the preparation of instant coffee. SCG is obtained from coffee processing where 45% of this by-product is generated in coffee beverage and instant coffee manufacturing (Cruz *et al*., 2014).

Coffee by-products such as SCG still contain a considerable amount of antioxidants and phenolic compounds such as rutin, catechin, quercetin, and others. The major polyphenol in coffee is known as chlorogenic acid (Wungrath *et al*., 2016). The antioxidant content and nutrient composition of the coffee and by-products vary depending on the processing, preparation and storage (Soares *et al*., 2015). A considerable amount of antioxidants remains in SCG after brewing and more attention has to pay in recycling the SCG for healthier development (Choi and Koh, 2017). The antioxidant plays roles to neutralize excess free radicals for preventing cells against free radical damage and contributing to the prevention of disease (Lobo *et al*., 2010). Yet, the antioxidant can be extracted in many other different techniques and one of them is enzyme-assisted extraction using pectinase, cellulase and others

*Corresponding author.
Email: *mkhairi@umt.edu.my*

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(Jooste *et al*., 2013).

Enzyme-assisted extraction seems to be a potential alternative to traditional solvent extraction methods and is gaining greater attention because it is an effective, sustainable and environmentally friendly extraction technology (Puri *et al*., 2012; Nadar *et al*., 2018). This method is typically used in leather making, skincare products, degumming of soybeans oil, juice processing and beer clarification (Puri *et al*., 2012). The role of the enzyme in the extraction is to destroy the cell wall matrix of the sample and especially to clarify the cloudy food product. The common enzyme that is used in this assisted extraction is pectinase, cellulase and hemicellulose (Jooste *et al*., 2013). Pectinases were known to be stable at pH 2.5-8.0 and active at a temperature below 50°C (Acufia-Argfielles *et al.*, 1995). The pH can affect the efficiency of extraction and the greatest yield extraction is always at the optimum pH value (Maimulyanti and Prihadi, 2014). Thus, pectinase contributes a lot in the food industry where it acts as juice clarifier, colour and yield improver in fruit mash treatment and used in degumming fibre crops (Singh *et al*., 2019).

The residues from the coffee industry are graded as highly pollutant due to the presence of organic material, specifically tannins, caffeine and polyphenols (Chanakya and Alwis, 2004). The major challenges of conventional extraction are time consuming and use a large amount of solvent (Ajila *et al*., 2011). Therefore, the purpose of this study was to determine the antioxidant activities and to measure the total phenolic and total flavonoid content along with the evaluation of antimicrobial activity in Arabica SCG extracted using pectinase at different pH values.

2. Materials and methods

Local Arabica (*Coffea arabica* L.) coffee was purchased from Kilang Kopi Fama, Banting (Selangor, Malaysia). The preparation of SCG was conducted using a French press coffee maker. The roasted coffee beans freshly were grounded using a coffee grinder for 90 s to a course-size ground. Hot water (98°C) of 500 mL was added to 40 g of coffee ground in the French coffee press before slowly pushed down the plunger after brewing for 2 mins. The coffee was poured out and the spent coffee ground (SCG) was collected to be dried out on the tray in the cabinet dryer for 24 hrs at 60°C. The collected dried SCGs were placed in ambient and dark containers in an airtight container prior to the extraction process.

2.1 Pectinase-assisted extraction

Arabica SCG of 8 g was added to a solution containing 0.8 mL of 0.2 M commercial pectinase in the liquid form and 120 mL of 200mM sodium acetate buffer in every pH 3, 4, 5, 6 and 7. The mixture was shaken gently in a water bath shaker at 37°C for 1 hr. The solution is then immediately placed in a water bath at 90°C for 5 min to stop enzymatic hydrolysis. Then, the solution was filtered using Whatman paper (no. 4) by vacuum pump to obtain the liquid. The liquid was concentrated in a rotary evaporator (Buchi Rotavapor R-200, New Hampshire, USA) at 60°C. The extract obtained was then kept in amber bottles and store in the chiller prior to further analysis (Zohdi and Amid, 2013).

2.2 Total phenolic content

The total phenolic compounds in SCG extracts was determined using Folin-Ciocalteu reagent according to the colourimetric method described by Ng *et al*. (2019). An aliquot (1 mL) of every sample was diluted into 50 mL of stock solution. From the stock solution, 1 mL was added to 17.9 mL of distilled water in and 0.5 mL of Folin-Ciocalteu reagent and left to stand for 1 min. Then, 1.5 mL of 20% sodium carbonate was added to the mixture. The sample prepared was then left at room temperature for 2 hrs in dark. The absorbance values were taken at 765 nm, resulting in mg GAE per gram of sample extract (mg GAE/g) expressed as gallic acid equivalent.

2.3 Total flavonoid content

The content of flavonoids in the extracts was determined using Hau *et al*. (2018) with slight modifications. Sample of 50 mg were mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1 M potassium acetate and 2.8 mL distilled water. The mixture was then incubated at room temperature for 30 mins. The absorbance of the reaction mixture taken at 415 nm. The result was expressed as mg of quercetin equivalent (QE) per gram of sample extract (mg QE/g).

2.4 Determination of antioxidative properties

2.4.1 Antioxidant activity based on 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The capacity of trapping of free radical DPPH was evaluated according to the method described by Malik *et al.* (2017) with slight modification. DPPH solution at the concentration of 6.1×10^{-5} M was prepared in ethanol. Diluted extract of 75 µL was mixed with 3 mL of the DPPH solution. After 1 hr, the absorbance was recorded at 515 nm using methanol with DPPH as negative control while quercetin as a positive control. All operations or conducted in dark or dim light. The inhibition percentage (IP) of the DPPH by the extract was calculated according to this formula:

IP (%) = [(A0 min - A60 min) / A0 min] x 100

Where A0 min is the absorbance of the blank at t = 0 min, and A60 min is the absorbance of samples at 60 mins. The result expressed as µmol Trolox equivalent (TE) per gram of sample on a dry basis, through a dose-response curve for Trolox (0-350 µM).

2.4.2 Ferric reducing antioxidant power (FRAP) analysis

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay by Chong *et al*. (2018) as a measure of antioxidant power. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (*v*/*v*/*v*). Three millilitres of the FRAP reagent was added to each test tube containing 0.1 mL diluted sample and mixed thoroughly. The absorbance value of the reacted mixture was observed at 593 nm after 0 min. The results were expressed as µmol trolox equivalent/g dried weight.

2.5 Antimicrobial activity

The well-diffusion method was used to determine the antimicrobial activity of SCG extract extracted with different pH. The SCG extract was tested on Muller-Hilton Agar (MHA) that has been swab with *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.* The well was prepared by punching the agar with a sterile micropipette tip (6 mm). Fifty microliters of the diluted extract at the concentration of 300mg/mL was introduced into the well and let it diffuse at room temperature. Gentamicin antibiotics were used as positive control while sterile distilled water was used as a negative control. The plates were incubated at 37°C for 24 hrs and the diameter of the inhibition zone was measured in centimetres (cm) (Duangjai *et al*., 2016).

2.6 Determination of individual flavonoids

The flavonoid standards that were used in HPLC determination were flavonol (quercetin, myricetin, kaempferol), catechin, flavones (apigenin, luteolin) and flavanone (naringin). Firstly, 5 mg of the samples were refluxed in 6 M HCl at 90°C for 2 hrs with 60% (v/v) aqueous methanol (Mohd Zainol *et al*., 2009). HPLC analyses were performed using an Analytical High - Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) with 4 solvent delivery system quaternary pump (LPG 3400 SD) equipped with a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 µl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim TM 120 C_{18} column (5 µm particle size, i.d. 4.6 x 250 mm). The stock solution of concentration 40 ppm was prepared by dissolving phenolic acid and flavonoids in 0.5mL HPLC-grade methanol (60%) followed by sonication for 10 min and the resulting volume was made up to one millimetre with the solvent for the mobile phase (acetonitrile and 1% aqueous acetic acid 1:9). The standard and working solutions were filtered through a 0.45 µm PVDR-syringe filter and the mobile phase was degassed before the injection of the solutions. The injection process in flow rate was adjusted to 0.7 mL/min with the column was thermostatically controlled at 28°C and the injection volume was kept at 20 µL Total running time was 31 mins and pre-equilibrium time was allowed at 5 mins. HPLC chromatograms were detected using a photodiode array UV detector at three different wavelengths (272, 280 and 310 nm) according to the absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions (Seal, 2016; Shin *et al*., 2019).

2.7 Statistical analysis

The statistical comparison was performed with one-way analysis (ANOVA) using Fisher's Least Significant Difference (LSD) test and values of $p < 0.05$ were considered significant. The statistical software used to analyze the data is Minitab 19.

3. Results and discussion

3.1 Extraction yield

Figure 1 shows that sample extract at pH 7 and pH 6 have a higher yield which is 52.96±1.88% and 51.48±5.99%, respectively. The yield of Arabica SCG extracts at pH 3 and pH 4 decreases due to the unfolding and decomposition of the enzyme structure in low acidic medium (Zohdi and Amid, 2013). The enzymes contain ionic groups where the active site is in a stable form. Therefore, the variation of a medium such as pH can change its ionic form that affects the reaction rate and the enzyme activity. Enzyme activity decreased at lower buffer pH when homogenizing with the sample which affects less enzyme solubilization in crude extract (Chen *et al*., 2006). In addition, the results obtained by Bravo *et al*. (2013) showed a similar trend to the study in which the yield of SCG extract increased as the pH increased. In pectin hydrolysis, pectinases group which consists of polygalacturonases and pectin lyase playing a relevant role in the hydrolysis.

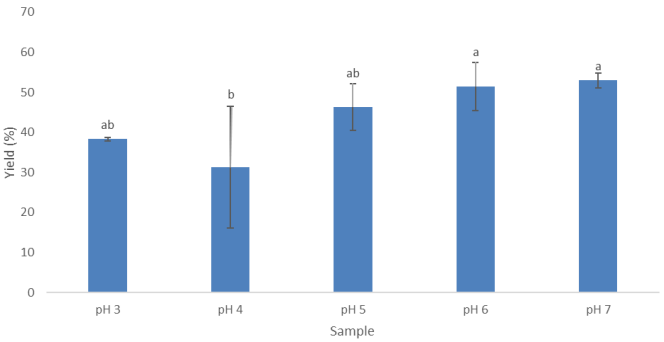

Figure 1. The yield of the Arabica SCG extract prepared from the SCG extracted using pectinase at different pH. Bars with the same letters are not significantly different (P<0.05).

3.2 Total phenolic content

Table 1 showed that SCG extracted using pectinase-assisted at pH 3, pH 4 and pH 5 had higher TPC value than that of pH 6 and pH 7. Since phenolic compounds were less vulnerable to oxidation at lower pH due to proton-protected hydroxyl groups, total phenolic compounds should be increased from pH 6 to pH 3 (Nayik and Nanda, 2016). The findings contrasted with the prediction that the enzymatic synthesis of polyphenolic compounds may result from the hydrolytic removal of polysaccharides from the plant cell wall, which retains phenolic compounds in the polysaccharide and lignin chains (Heemann *et al*., 2019). However, higher phenolic content in extracts could be associated with increased disruption and maceration due to enzymatic pre-treatment, which contributes to the release of bound phenolic (Mushtaq *et al*., 2015).

3.3 Total flavonoid content

Table 1 also showed that the total flavonoid content (TPC) of SCG extracted using pectinase-assisted pH 5 had significantly ($p<0.05$) the highest TPC (22.75 ± 0.27 mg QE / g). This data corresponds with the study made on saffron honey that showed the value of TFC was higher at pH 4.5 mg QE/ 100 g and dropped at pH 6 to 9.46 mg QE/100 g (Nayik and Nanda, 2016). Then, the author also claimed that the de-protonation of the hydroxyl group caused the decreased oxidation of honey polyphenols at low pH in TFC analysis. This was also supported by Haile and Kang (2019), as they expressed the fermentation of coffee influence the flavonoid content in the coffee.

3.4 Antioxidant activities

3.4.1 DPPH radical scavenging assay

SCG samples extracted using pectinase-assisted technique at pH 4 showed the strongest inhibition of 2.2-diphenyl-2-picrylydrazyl (DPPH) radicals, which was significantly higher than other samples (Table 1). The finding is in concert with the study by Zhu *et al*. (1997) who stated that green tea catechin is very stable at pH < 4 for 18 hrs while unstable at pH > 8 which degraded completely in a few minutes. They also stated that the green tea catechin at pH 6.5 to 7.5 was less stable compared to the acidic medium. Low pH is the main aspect of extraction efficiency as the flavanol is stable below pH 5. The structural alteration of the flavonoid decreases with the decreasing pH that leads to the opening of structures that are normally inaccessible (Zimmermann and Gleichenhagen, 2011). MEBP powder samples encapsulated with 8% GA exhibited the best radical scavenging activity among all those which, due to the good condition or non-clotting state of the microencapsulated powder, were able to fully demonstrate its antioxidant capacity.

3.4.2 Ferric reducing antioxidant power (FRAP)

Table 1 also illustrates that all pectinase extract with different pH values had a higher value than the negative control (distilled water). Moreover, extraction at pH 3, pH 4, pH 5 and pH 6 showed higher inhibition compared to pH 7. Owusu-Apenten (2015) postulated that at pH higher than 7.0) or lower than 3, would decreased the rate of reduction of Fe (III) resulting in an incomplete response At high pH values, the ascorbic acid or high antioxidant sample act to iron (III)-OH formation and

Table 1. Total phenolic content (TPC), total flavonoid content (TFC), FRAP assay and DPPH inhibition of the SCG extracted using pectinase at different pH

Samples	TPC (mg GAE/g sample)	TFC of dried plant material (mg QE/g)	DPPH radical inhibition (%)	Ferric reducing antioxidant power (FRAP) assay
pH 3	61.38±5.06^{a}	15.10±3.11^{c}	40.56±2.21^{d}	0.04±0.001^{ab}
pH 4	42.75±1.26^{b}	18.62±1.11^{bc}	55.37±1.36^{b}	0.05±0.001^{a}
pH 5	54.13±5.22^{a}	22.76±5.22^{a}	34.84±2.42^{d}	0.05±0.001^{a}
pH 6	32.38±2.04^{c}	19.53±1.14^{ab}	45.06±3.31^{c}	0.04±0.001^{ab}
pH 7	37.54±2.68^{b}	21.60±2.23^{ab}	34.76±2.57^{e}	0.03±0.001^{b}
blank	-		9.78±0.57^{f}	0.01±0.001^{c}
Trolox	-		68.74±3.17^{a}	-

Values represent the mean±standard deviation. Values with the same superscript letters within the same column are not significantly different (P<0.05).

decreasing the rate of reduction for the Fe (III) hydroxyl complex while at lower pH value, the protonation of ferrozine occurred as the reduction of Fe (III) decreasing with pH (Jaselskis and Nelapaty, 1972). Most researchers used pH 4 and pH 5 with 50°C and 40°C as the optimal pH and temperature in preparation for enzyme - assisted extraction (Nguyen *et al*., 2014; Wang *et al*., 2017; Ghandahari Yazdi *et al*., 2019). The antioxidant capacity obtained is highly dependent on the concentration of phenolic compounds of the samples, the type of enzymes and type of extraction, respectively.

3.5 Antimicrobial activities

There was no significant difference in the growth of the inhibition zone for *B. subtilis,* yet *S. aureus* and *P. aeruginosa* showed somewhat significant difference between the samples especially at pH 7 (Table 2). Table 2 also tabulates that Arabica SCG extract at pH 7 has the most significant inhibition effect than gentamicin (antibiotic) on *S. aureus* and *P. aeruginosa* which are 2.17±0.15 cm and 2.77±1.10 cm while the antibiotics only 1.23±0.25 cm and 1.9±0.0 cm, respectively.

S. aureus, a gram-positive and *P. aeruginosa*, a gram -negative bacteria were more susceptible to the SCG extract especially at pH 7 where a bigger region of inhibition zone on the agar. The alteration of pH can affect the growth of bacteria where most of the optimum pH of most bacteria is at pH 7. Meanwhile, *E. coli* shows not susceptible to every sample except for gentamicin might because these bacteria have a structure of the bacterial envelope or this concentration of the extract is not strong enough to against the bacteria. This is in accordance with the study by Klangpetch (2017) that *E. coli* showed an inhibition zone only when the SCG extracted with 60% and 100% of ethanol. The caffeine concentration of more than 1% is excellent in lowering or inhibiting the growth of *E. coli*, below that there is no effect on bacterial growth (Ramanaviciene *et al*., 2003). Radha Krishnan *et al*. (2014) stated that the antimicrobial activity related to the antioxidant activity like phenolic compounds in plant sources could inhibit the pathogenic bacteria. This compound can break down the cell wall of bacteria, disrupt the cytoplasmic membrane, and disturb protein translocation and others (Shan *et al*., 2007).

3.6 Determination of individual flavonoid using high-performance liquid chromatography (HPLC)

Figure 2 shows the standard flavonoid HPLC chromatogram identified by its retention time and by spiking under the same conditions with standards. Figure 3 shows that every peak on the chromatogram indicates the presence of individual flavonoids present during specific retention time. This can be detected if more time and flavonoid standard is allowed to run the sample (Gandhiraja *et al*., 2009). Table 3 shows that most of the flavonoids identified in the study are the same as the rutin, myricetin, kaempferol, gallic acid, catechin, gallate epigallocatechin, quercetin and p-coumaric acid used. The epigallocatechin gallate exhibited the highest concentration in every sample ranging from 1638±406 to 5293±6805 mg/100g while the lowest flavonoid concentration in every sample is gallic acid that ranging from 91.22±2.21 to 760.8±98.8 mg/100 g.

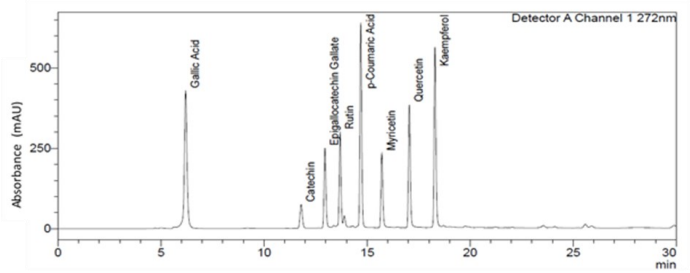

Figure 2. HPLC Chromatogram of flavonoids of the standard mixture

The results also showed that SCG extract in pH 7 had the highest concentration of total individual flavonoid while pH 4 shows the lowest concentration of total individual flavonoid between samples. The data obtained had supported the result from the antimicrobial activity where large inhibition occurs at SCG in pH 7

Table 2. Inhibition effect of Arabica SCG extracted using pectinase at different pH against pathogenic bacteria in antimicrobial activity

Sample	Inhibition Zone (cm)			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*
pH 3	1.08±0.07^{b}	1.93±0.10^{b}	1.87± 0.23^{b}	No inhibition zone
pH 4	0.7±0.6^{b}	1.55±0.06^{c}	1.67±0.21^{b}	
pH 5	1.07±0.05^{b}	1.63±0.09^{c}	2.00± 0.1^{ab}	
pH 6	1.07±0.15^{b}	2.05±0.05^{ab}	2.23±0.21^{ab}	
pH 7	1.1±0.1^{b}	2.17±0.15^{a}	2.77±1.10^{a}	
Gentamicin	2.23±0.25^{a}	1.23±0.25^{d}	1.9± 0.00^{b}	2.00± 0.02a

Values represent the mean±standard deviation. Values with the same superscript letters within the same column are not significantly different (P<0.05). Gentamicin was used as the standard antibiotic for comparison.

Table 3. The concentration of individual flavonoid in Arabica SCG extracted using pectinase at different pH identified by the HPLC system

Flavonoid compound	The concentration of the individual flavonoid in every sample (mg/100 g)				
	pH 3	pH 4	pH 5	pH 6	pH 7
Catechin	641.07±44.52^{a}	1151.87±88.76^{ab}	1874.67±153.53^{ab}	2044.98±227.54^{a}	5112.76±66.43^{a}
Epigallocatechin Gallate	5293.67±68.05^{a}	1911.32±112.21^{a}	1638.56±40.56^{ab}	1776.54±171.92^{a}	1936.72±78.54^{b}
Gallic Acid	120.16±11.54^{a}	91.22±2.21^{c}	182.7±12.41^{ab}	104.4±9.81 ^{a}	760.84±98.83^{c}
Kaempferol	585.98±19.28^{a}	1092.95±25.55^{abc}	1611.40±464.34^{ab}	1800.43±24.67^{a}	570.88±24.05^{c}
Myricetin	897.09±24.93^{a}	1696.77±47.78^{a}	923.36±84.9^{ab}	790.48±43.19^{a}	1552.54±30.84^{b}
p-Coumaric acid	299.31±5.42^{a}	502.33±47.43^{bc}	262.61±76.62^{ab}	535.22±31.64^{a}	671.63±33.54^{c}
Quercetin	975.67±83.70^{a}	501.08±70.73^{bc}	1480.89±128.19^{ab}	407.52±37.29^{a}	558.62±14.28^{c}
Rutin	1555.68±49.69^{a}	1659.26±32.41^{a}	2310.75±576.54^{a}	1864.57±121.06^{a}	948.22±19.52^{c}

Values represent the mean±standard deviation. Values with the same superscript letters within the same row are not significantly different (P<0.05).

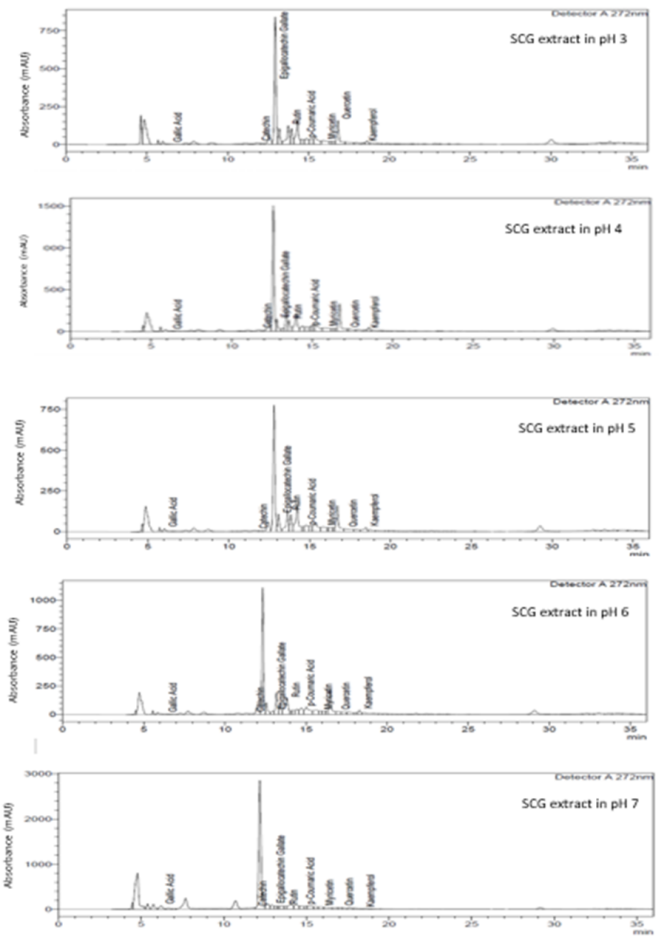

Figure 3. HPLC chromatogram of the SCG extracted using pectinase-assisted extraction at pH 3, pH 4, pH 5, pH 6, pH 7.

because containing a high amount of individual flavonoid. Flavonoids such as quercetin and catechin may be the compounds responsible for antiviral, antiallergic, antitumor, antibacterial, anti-fungal, and antithrombotic activity expressing anti-infective and anti -replicative characteristics against the herpes virus, parainfluenza virus as well as inhibiting the growth of *P. aeruginosa* and *S. aureus* (Middleton, 1996; Kaurinovic and Vastag, 2019). The amount of gallic acid (Table 3) found in this study was in line with Ghandahari Yazdi *et al*. (2019) who reported gallic acid found in pistachio green hull using pectinase and cellulase assisted extraction is lower than 5.70±0.02 and 4.69±0.01 mg/g dry extract powder respectively as the pectinolytic and cellulolytic enzyme treatment had affected the phenolic composition and antioxidant activity of the food product.

4. Conclusion

The extraction of Arabica SCG using pectinase at different pH had a significant effect on antioxidant and antimicrobial properties. Low pH showed better antioxidant properties, while higher pH showed better antimicrobial properties. The SCG sample extracted at pH 4 showed the best antioxidant properties in DPPH and FRAP. Extraction at pH 7 clearly showed better antimicrobial activity with the largest inhibition zone for *P. aerunogisa* and *S. aureus*. The HPLC analysis showed the presence of flavonoids, namely quercetin, kaempferol, rutin, gallic acid, catechin, epigallocatechin gallate, p-coumaric acid and myricetin, in all samples with different concentrations. The study showed that the optimum pH for the extraction of Arabica SCG was at pH 4 to 5 which provided the highest yield in antioxidant capacity and activity that could be used for food preservation as well as in the pharmaceutical industry.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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